Amendments to the Specification as Represented in PCT Application WO 2004/044247 Published On 27 May 2004:.

Please insert the following paragraph at page 1, line 3:

-- **Cross Reference to Related Applications**

This application is the national stage of PCT/IB2003/005312 filed 3 November 2003, which claims the benefit of United States Provisional Application Number 60/425,327 filed on November 12, 2002. --

Please replace the paragraph beginning at page 2, line 3 (This is paragraph [0005] of corresponding Patent Application Publication US2006/0257871. The bracketed paragraphs "([])" shown in these Amendments to the Specification... here and below are from PAP '871 and are included for the Examiner's ease of reference.), with the following amended paragraph:

--These probes are useful in hybridizing to RNA amplified by the Reverse Transcriptase Polymerase Chain Reaction (RT PCR). RT-PCR is a powerful ribonucleic acid amplification technique that can be used for the detection of small numbers of ribonucleotide acid targets from bacteria and/or from fungus-yeast whose ~~in vitro~~ *in vitro* cultivation is difficult or lengthy. RT PCR requires the presence of living specimens for detection. In its simplest form, RT PCR is an in vitro method for the enzymatic synthesis of specific cDNA sequences. Using ~~one~~ oligonucleotide primers that hybridize to an RNA strand and flank the region of interest ~~in the target cDNA~~, several cDNA sequences are synthesised by Reverse Transcriptase. A repetitive series of cycles involving template denaturation, primer annealing, and extension of annealed primers by DNA polymerase results in the exponential accumulation of a specific fragment whose termini are defined by the 5' ends of the primers. PCR ~~produce~~ produces a selective enrichment of a specific DNA sequence by a factor of ~~10.sup.12~~ 1×10^{12}. The PCR method is described in Saiki et al, 1985, Science 230:1350 and is the subject of U.S. Pat. Nos. 4,683,195, 4,683,202 and 4,800,159 (these references are incorporated herein by reference). This method has been used to detect the presence of the aberrant sequence in the beta-globin gene which ~~is related to~~ causes sickle cell anemia (Saiki et al., 1985, supra) and the human immunodeficiency virus (HIV) RNA (Byrne et al., 1988, Nuc.Acids Res. 16:4165) --

Please replace the paragraph beginning at page 3, line 3 ([0007]), with the following amended paragraph:

--It is clear that a rapid diagnostic method, less than 24 hours, for detecting bacteria and fungus-yeast in industrial samples with the same sensitivity as ~~culture~~ culturing would be a significant improvement over currently used methods.--

Please replace the paragraph beginning at page 3, line 9 ([0008]), with the following amended paragraph:

--The present invention pertains to methods and reagents for the rapid detection of bacteria and fungus -yeast in sterile and non sterile ~~product~~ products in less than 24 hours. In a preferred embodiment, a target region from a one-step Reverse Transcriptase Polymerase Chain Reaction of RNA and the resultant amplified DNA is treated with probes which can hybridize to the amplified DNA of bacteria or fungus-yeast but not other organisms (mammalian, plant, insects ...) or ~~virus~~ viruses.--

Please replace the paragraph beginning at page 3, line 15 ([0010]), with the following amended paragraph:

--The Tth DNA polymerase is a thermostable enzyme with RNA-dependent Reverse Transcriptase activity and with DNA-dependent Polymerase activity, allowing the combination of RT and PCR in a single-tube reaction resulting in a faster analysis of the presence of RNA from bacteria or fungus-yeast.--

Please replace the paragraph beginning at page 3, line 19 ([0011]), with the following amended paragraph:

--Using one-step Real-time Reverse Transcriptase Polymerase Chain Reaction, the invention ~~enable~~ enables the user to perform a rapid RT -PCR and simultaneously detect and quantify the presence of RNA from bacteria and/or fungus-yeast by monitoring fluorescence during real time polymerase chain reaction amplification ~~with~~ without any risk of false ~~positive~~ positives due to opening the tube between the RT and PCR steps and from possible PCR product environmental contamination due to precedent amplification reactions in the laboratory.--

Please replace the paragraph beginning at page 4 ([0012]), first paragraph beginning on line 3, with the following amended paragraph:

--The methods of the present invention thus enable determination of the presence of bacteria and/or fungus-yeast more rapidly than technologies ~~with~~ using prior art detection methods.--

Please replace the paragraph beginning at page 4, line 6 ([0013]), with the following amended paragraph:

--Using one-step Real-time Reverse Transcriptase Polymerase Chain Reaction, the invention ~~enable~~ enables the user to perform a rapid RT -PCR and simultaneously analyze and quantify the presence of RNA from bacteria and/or fungus-yeast by monitoring fluorescence during real time polymerase chain reaction amplification ~~with~~ without any risk of false ~~positive~~positives due to opening the tube between the RT and PCR steps and from possible PCR product environmental contamination due to precedent amplification reactions. The basic RT PCR process is carried out as follows.--

Please replace the paragraph beginning at page 4, line 14 ([0015]), with the following amended paragraph:

--A sample is provided which needs to be tested or which is suspected of ~~contain~~ containing a particular ribonucleic acid sequence of interest, the "target sequence." The ribonucleic acid contained in the sample may be first reverse transcribed into cDNA (using an enzyme like Tth DNA polymerase as purified enzyme and [[a]]an oligonucleotide or peptide nucleic acid PNA), and then denatured, using physical means, which are known to those of skill in the art. A preferred physical means for strand separation involves heating the nucleic acid until it is completely (>99%) denatured. Methods for the amplification of RNA targets using a thermostable DNA polymerase are described in PCT/US90/07641, filed Dec. 21, 1990, and incorporated herein by reference.--

Please replace the paragraph beginning at page 7, line 14 ([0028]), with the following amended paragraph:

--(a) ~~extract~~ extracting bacteria or fungus-yeast ribonucleic acid (RNA) from the sample up to 1000 ml by centrifiltration on membranes and/or DEAE resin ~~followingfollowed~~ by incubation with DNAse.--

Please replace the paragraph beginning at page 8, line 6 ([0030]), with the following amended paragraph:

--(c) ~~amplified~~ amplifying the cDNAs formed to a detectable level by the Polymerase Chain Reaction with said polymerase enzyme like Tth DNA polymerase and polynucleotide primers and probes with a nucleotide sequence selected from the group consisting of:

Seq ID No 1 TGGAGCATGTGGTTTAATTCGA	[primer forward]
Seq ID No 2 TGCGGGACTTAACCCAACA	[primer reverse]
Seq ID No 3 AGAGTTTGATCATGGCTCAGA	[primer forward]
Seq ID No 4 TTACCCCACCTACTAGCTAAT	[primer reverse]
Seq ID No 5 GYGGAGCATGTGGYTTAATTCG	[primer forward]
Seq ID No 6 TTGCGCTCGTTRCGGGACTT	[primer reverse]
Seq ID No 7 GGGAAACTCACCAGGTCCA	[primer forward]
Seq ID No 8 CGTTATCGCAATTAAGCAGACA	[primer reverse]
Seq ID No 9 GGTAACGGGGAATWAGGGTTC	[primer forward]
Seq ID No 10 TTGGGTAATTTGCGCGCCTG	[primer reverse]
Seq ID No 11 TGCATGGYTGTCGTCAGCTCGTG	[probe forward]
Seq ID No 12 GAGTGGCGGACGGGTGAGTAA	[probe forward
Seq ID No 13 ACAGGTGGTGCATGGTTGTC	[probe forward]
Seq ID No 14 TCAGCTCGTGTCGTGAGATGTT	[probe forward]
Seq ID No 15 ACAGGTGCTGCATGGCTGTC	[probe forward]
Seq ID No 16 TCAGCTCGTGTTGTGAAATGTT	[probe forward]
Seq ID No 17 AGGATTGACAGATTGAGAGCTCTT	[probe forward]
Seq ID No 18 CGGAGAGGGAGCCTGAGAA	[probe forward]
Seq ID No 19 CGGCTACCACATCCAAGGAA	[probe forward]

Please replace the paragraph beginning at page 9, line 1 ([0031]), with the following amended paragraph:

--The cDNA target sequence can be synthesized by Reverse Transcriptase activity of ~~Tth~~ *Tth*, or an enzyme like ~~Tth~~ *Tth*, and is amplified by the DNA-dependent Polymerase activity of the DNA polymerase in the same tube by means of one step real time RT-PCR.--

Please replace the paragraph beginning at page 9, line 18 ([0034]), with the following amended paragraph:

--The invention may also be practiced with a composition for detecting bacteria which comprises ~~[[a]]~~ polynucleotide primers and ~~a probe~~probes consisting of the sequence:

Seq ID No 5 GYGGAGCATGTGGYTTAATTCG	[primer forward]
Seq ID No 6 TTGCGCTCGTTRCGGGACTT	[primer reverse]
Seq ID No 13 ACAGGTGGTGCATGGTTGTC	[probe forward]
Seq ID No 14 TCAGCTCGTGTCGTGAGATGTT	[probe forward]
Seq ID No 15 ACAGGTGCTGCATGGCTGTC	[probe forward]
Seq ID No 16 TCAGCTCGTGTTGTGAAATGTT	[probe forward]--

Please replace the paragraph beginning at page 10, line 1, with the following amended paragraph:

--The invention also concerns the above mentioned method and kit wherein the composition for detecting fungus-yeast comprises [[a]] polynucleotide primers and a probe consisting of the sequence:

Seq ID No 7 GGGAAACTCACCAGGTCCA	[primer forward]
Seq ID No 8 CGTTATCGCAATTAAGCAGACA	[primer reverse]
Seq ID No 17 AGGATTGACAGATTGAGAGCTCTT	[probe forward]--

Please replace the paragraph beginning at page 10, line 8 ([0036]), with the following amended paragraph:

--Alternatively, the composition for detecting fungus-yeast comprises ~~[[a]]~~ polynucleotide primers and ~~a probe~~ probes consisting of the sequence:

Seq ID No 9 GGTAACGGGGAATWAGGGTTC	[primer forward]
Seq ID No 10 TTGGGTAATTTGCGCGCCTG	[primer reverse]
Seq ID No 18 CGGAGAGGGAGCCTGAGAA	[probe forward]
Seq ID No 19 CGGCTACCACATCCAAGGAA	[probe forward]--

Please replace the paragraph beginning at page 12, line 23 ([0053]), with the following amended paragraph:

--In these precedent patents, universal bacterial probes are ~~using~~ used only for positive controls. Universal primers for bacterial- fungus-yeast are ~~using~~ used for identification after cloning and sequencing of the amplified product or hybridization on a DNA chip. The ~~used~~ use of rRNA targets for sterility controls for detection of alive bacteria and fungus-yeast in sterile or non sterile industrial ~~product~~ products has not been described before this invention.--

Please replace the paragraph beginning at page 14, line 10 ([0058]), with the following amended paragraph:

--A preferred method for analysis of samples is by single filtration (for filterable liquids). ~~Specificity of~~ Specifically, extraction ~~from~~ of bacteria or fungus-yeast ribonucleotide from the sample ~~up to 1000 mL~~ (up to 1000 mL) is done by centrifiltration ~~following~~ followed by an incubation with DNase.--